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Somatostatin Depresses the Excitability of Subicular Bursting Cells: Roles of Inward Rectifier K⁺ Channels, KCNQ channels and Epac

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Abstract

The hippocampus is a crucial component for cognitive and emotional processing. The subiculum provides much of the output for this structure but the modulation and function of this region is surprisingly under-studied. The neuromodulator somatostatin (SST) interacts with five subtypes of SST receptors (sst₁ to sst₅) and each of these SST receptor subtypes is coupled to Gi proteins resulting in inhibition of adenylyl cyclase (AC) and decreased level of intracellular cAMP. SST modulates many physiological functions including cognition, emotion, autonomic responses and locomotion. Whereas SST has been shown to depress neuronal excitability in the subiculum, the underlying cellular and molecular mechanisms have not yet been determined. Here, we show that SST hyperpolarized two classes of subicular neurons with a calculated EC₅₀ of 0.1 μ M. Application of SST (1 μ M) induced outward holding currents by primarily activating K⁺ channels including the G-protein-activated inwardly-rectifying potassium channels (GIRK) and KCNQ (M) channels, although inhibition of cation channels in some cells may also be implicated. SST-elicited hyperpolarization was mediated by activation of sst₂ receptors and required the function of G proteins. The SST-induced hyperpolarization resulted from decreased activity of AC and reduced levels of cAMP but did not require the activity of either PKA or PKC. Inhibition of Epac2, a guanine nucleotide exchange factor, partially blocked SST-mediated hyperpolarization of subicular neurons. Furthermore, application of SST resulted in a robust depression of subicular action potential firing and the SST-induced hyperpolarization was responsible for its inhibitory action on LTP at the CA1-subiculum synapses. Our results provide a novel cellular and molecular mechanism that may explain the roles of SST in modulation of subicular function and be relevant to SST-related physiological functions.

Keywords

Peptide; Subiculum; Glutamate; neuromodulation; synaptic transmission

Introduction

Somatostatin (somatotropin release inhibiting factor; SST) is a cyclic poly peptide that serves as a neurotransmitter or neuromodulator in the CNS. There are two biologically active isoforms of SST, SST-14 and SST-28. Both SST isoforms can similarly activate all the five cloned G_i-protein-coupled receptors (sst₁₋₅) (Martel et al., 2012; Patel, 1999; Theodoropoulou and Stalla, 2013). Activation of the five SST receptor subtypes inhibits adenylyl cyclase (AC), resulting in a decreased level of cAMP production and inhibition of protein kinase A (PKA) (Patel, 1999), although SST receptors also couple to Epac (the Exchange Protein Directly Activated by cAMP) which is a guanine nucleotide exchange factor and includes Epac1 and Epac2 (Jacobs et al., 2010). SST-immunoreactivity has been detected at high levels in several brain regions, including the mediobasal hypothalamus and median eminence, amygdala, preoptic area, hippocampus, striatum, cerebral cortex, sensory regions and the brain stem (Epelbaum, 1986; Martel et al., 2012; Viollet et al., 2008), where it colocalizes with GABA and is released by a subpopulation of GABAergic interneurons. In addition to its initial role as an inhibitor for growth hormone secretion from the anterior pituitary somatotroph cells, SST modulates many physiological functions such as cognition, emotion, autonomic responses and locomotion as well as several pathological disorders including anxiety, depression, schizophrenia and epilepsy (Martel et al., 2012). However, the cellular and molecular mechanisms underlying SST-mediated modulation of these physiological functions and pathological disorders have not fully been determined.

The subiculum is the major output region of the hippocampus and targets a variety of cortical and subcortical areas (O'Mara et al., 2001). Together with CA1 pyramidal neurons, the subiculum processes sensory and motor cues to form a cognitive map encoding spatial, contextual, and emotional information, which are then transmitted throughout the brain. Subiculum is therefore an integral component in the networks underlying memory for spatial tasks (O'Mara et al., 2001). In the hippocampus, SST hyperpolarizes CA1 pyramidal neurons and reduces action potential (AP) firing frequency (Moore et al., 1988; Pittman and Siggins, 1981), possibly via activation of KCNK-like leak K⁺ channels (Schweitzer et al., 1998). SST increases KCNQ channel currents (M-currents) (Moore et al., 1988; Qiu et al., 2008; Schweitzer et al., 1998) and produces a hyperpolarization-independent reduction in AP firing by increasing the membrane insertion of G-protein-activated inwardly rectifying K⁺ (GIRK) channels (Lucas and Armstrong, 2015). In addition, SST inhibits excitatory synaptic transmission by reducing glutamate release (Tallent and Siggins, 1997) and depresses epileptiform bursting and seizure activity (Qiu et al., 2008; Tallent and Siggins, 1999). In contrast to the relatively comprehensive studies on the roles and mechanisms of SST in modulating neuronal network activities in the hippocampus, the effects of SST on neuronal activities in the subiculum have not fully been determined, although SST has been shown to hyperpolarize both bursting cells (BCs) and regular firing cells (RCs) (Greene and Mason, 1996a; Greene and Mason, 1996b) and reduce both GABA_A receptor-mediated fast IPSPs and GABA_B receptor-induced slow IPSPs in the subiculum (Greene and Mason, 1996b). However, the ionic and signaling mechanisms whereby SST depresses neuronal excitability in the subiculum are completely unknown. Determining the mechanisms underlying SST-elicited modulation of neuronal excitability in the subiculum is important

because the subicular neurons and CA1 pyramidal neurons have distinct intrinsic properties. For example, almost all the CA1 pyramidal neurons are RCs whereas more than 75% of the subicular neurons are BCs and a small population of subicular neurons are RCs (Mason, 1993; Mattia et al., 1993). We therefore studied the effects of SST on neuronal activities in the subiculum and our results demonstrate that SST hyperpolarizes subicular neurons via ssr_2 -mediated inhibition of AC and Epac2. We also demonstrate that SST depresses neuronal excitability by activation of GIRK and KCNQ channels and inhibition of cation channels. SST-mediated hyperpolarization decreases the magnitude of long-term potentiation (LTP) in the subiculum.

Methods

Slice preparation

Horizontal brain slices (350 μ m) were prepared from Sprague-Dawley rats (21-35 days old) as described previously (Zhang et al., 2015) with slight modification (Xiao et al., 2014). After being deeply anesthetized with isoflurane, animals were decapitated and their brains were dissected out in ice-cold saline solution that contained (in mM) 130 N-methyl-D-glucamine (NMDG)-Cl, 24 NaHCO₃, 3.5 KCl, 1.25 NaH₂PO₄, 0.5 CaCl₂, 5.0 MgCl₂, and 10 glucose, saturated with 95% O₂ and 5% CO₂ (pH 7.4, adjusted with HCl). Slices were then incubated in the above solution except NMDG-Cl was replaced with NaCl at 35°C for 1 h for recovery and kept at room temperature (~24°C) until use. All animal procedures conformed to the guidelines approved by the University of North Dakota Animal Care and Use Committee.

Recordings of APs, resting membrane potentials and holding currents from subicular neurons

Whole-cell patch-clamp recordings using a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA) in current- or voltage-clamp mode were made from the pyramidal neurons in the subiculum visually identified with infrared video microscopy (Olympus BX51WI) and differential interference contrast optics. During recordings, the bath temperature was maintained at 30-32°C by a temperature controller (TC-324C, Warner Instruments). The recording electrodes were filled with (in mM) 100 K⁺-gluconate, 8 KCl, 2 MgCl₂, 40 HEPES, 0.6 EGTA, 2 ATPNa₂, 0.4 GTPNa and 7 phosphocreatine (pH 7.3), unless stated otherwise. The extracellular solution comprised (in mM) 130 NaCl, 24 NaHCO₃, 3.5 KCl, 1.25 NaH₂PO₄, 2.5 CaCl₂, 1.5 MgCl₂ and 10 glucose, saturated with 95% O₂ and 5% CO₂ (pH 7.4). Data were filtered at 2 kHz, digitized at 10 kHz, acquired on-line and analyzed after-line using pCLAMP 10.4 software (Molecular Devices, Sunnyvale, CA). After the formation of whole-cell configuration, we injected currents from -100 pA to 400 pA (duration: 400 ms) at a step of 50 pA with an interval of 10 s/injection to identify the recorded neurons. BCs fire an early burst of 2-4 APs upon injection of depolarizing current of threshold intensity, whereas in RCs, prolonged depolarization causes either a single AP or trains of single spikes (Mason, 1993; Mattia et al., 1993). SST was dissolved in the extracellular solution and bath applied to the slice. To avoid potential desensitization induced by repeated applications of the agonist, one slice was limited to only one application of SST. Frequency of APs was calculated by Mini Analysis 6.0.1 (Synaptosoft Inc., Decatur,

GA). Resting membrane potentials (RMPs) and holding currents (HCs) at -60 mV were recorded in the extracellular solution supplemented with TTX (0.5 μ M), kynurenic acid (1 mM) and picrotoxin (100 μ M) to block glutamatergic and GABAergic transmission. I-V curves were obtained by using a ramp protocol from -120 mV to -40 mV at a speed of 0.045 mV/ms. We compared the I-V curves recorded before and during the application of SST for 3-5 min when its effect was maximal.

Recordings of synaptic currents and LTP from subicular pyramidal neurons

For the recordings of AMPA EPSCs and LTP, the above-mentioned K^+ -gluconate internal solution was used and the extracellular solution was supplemented with picrotoxin (100 μ M) to block GABAergic transmission. The holding potential was at -60 mV and the stimulation frequency was 0.1 Hz. A stimulation electrode was placed in the alveus of the border of CA1 and subiculum and recordings were then conducted from the neurons in the middle part of the subiculum (Wozny et al., 2008). Because the predominant neuronal type in the subiculum was BCs, we limited our experiments to this neuronal type unless stated otherwise. After recording stable basal AMPA EPSCs at -60 mV (0.1 Hz) for 5 min, SST (1 μ M) or saline (0.9% NaCl) dissolved in the extracellular solution was perfused for 5 min, which is when the maximal effect of SST was usually observed. An induction protocol of high-frequency stimulation (HFS, 100 Hz for 1 s, repeated 4 times at an interval of 10 s, in current clamp at the RMP) was then applied to induce LTP. Recording of AMPA EPSCs at -60 mV (0.1 Hz) was then resumed to observe the expression of LTP.

Data analysis

Data are presented as the means $\pm$ S.E.M. SST concentration-response curve was fit by using the Hill equation: $I = I_{\max} \times \{1/(1 + (EC_{50}/\text{ligand})^n)\}$, where $I_{\max}$ is the maximum response, EC_{50} is the concentration of ligand producing a half-maximal response, and n is the Hill coefficient. Student's paired or unpaired t test or analysis of variance (ANOVA) was used for statistical analysis as appropriate; P values are reported throughout the text and significance was set as $P < 0.05$.

Chemicals

SST was purchased from Bachem. The following chemicals were products of R&D Systems: tetrodotoxin (TTX), kynurenic acid, picrotoxin, CGP55845, BAPTA, SCH23390, linopirdine, ZD7288, CH275, L-803,087, BIM 23052, $1R,1'S,3'R/1R,1'R,3'S$ -L-054,264 (abbreviated as L-054,264), octreotide, CYN154806, GDP- β -S, MDL12330A, SQ22536, Rp-cAMPS, H89, KT5720, GF109203X, forskolin, IBMX, HJC0350. PKI(5-24) was provided by Enzo Life Sciences. Drugs were initially prepared in stock solution, aliquoted and stored at -20°C . For those chemicals which are only soluble in dimethyl sulfoxide (DMSO), the concentration of DMSO was less than 0.1% . This concentration of DMSO either in the recording pipettes or in the bath had no significant effects on neuronal activity.

Results

SST reduces the excitability of two classes of subicular neurons

We initially studied the effects of SST on HCs recorded at -60 mV from subicular neurons. Subiculum was distinguished from bordering regions by the diffuse distribution of pyramidal cells, compared to the tightly packed pyramidal cell layer of CA1, and the lack of distinct cortical layers in the entorhinal cortex (Fig. 1). The extracellular solution contained TTX (1 μ M), kynurenic acid (1 mM), picrotoxin (100 μ M) and CGP55845 (1 μ M) to block synaptic transmission. Under these circumstances, the effects of SST should only reflect changes to the recorded neurons. Because pyramidal neurons in the subiculum are electrophysiologically classified as either BCs or RCs (Graves et al., 2012; Wozny et al., 2008), we identified the neuronal types by direct current injections to the recorded cells after initial formation of the whole-cell configuration (Fig. 2). BCs fire an early burst of 2-4 APs upon injection of depolarizing current of threshold intensity, whereas in RCs, prolonged depolarization causes either a single AP or trains of single spikes (Mason, 1993; Mattia et al., 1993). Of the 69 cells recorded, 58 cells were classified as BCs (84%) and 11 cells were categorized as RCs (16%). The ratio of BCs to RCs was generally consistent with those reported previously showing that BCs account for 75% to 100% of the subicular cells (Mason, 1993; Mattia et al., 1993). Application of SST (1 μ M) induced an outward HC in both BCs (31.7 ± 26.4 pA, $n = 58$, $P < 0.0001$, Fig. 2A-C) and RCs (24.0 ± 14.4 pA, $n = 11$, $P < 0.001$, Fig. 2B-C). The reversibility of SST-induced increases in HCs varied considerably among cells. For most of the cells, the response of the SST was only partially recovered after wash in SST-free extracellular solution for 20 min. However, there were a portion of the cells which exhibited either complete recovery or persistent increase in outward HCs after wash. Because the predominant neuronal type in the subiculum are BCs and since there was no significant difference ($P = 0.24$) for SST-induced outward HCs between BCs and RCs (Fig. 2C), we recorded from the BCs for the remaining experiments to identify the underlying mechanisms. The EC_{50} of the SST was measured to be 0.1 μ M by using the data recorded from BCs (Fig. 2D). We used SST at 1 μ M for further experiments because this is a near-saturating concentration.

We recorded RMPs of the BCs in response to SST application in current-clamp. Application of SST hyperpolarized BCs significantly (Control: -59.4 ± 0.8 mV, SST: -61.3 ± 0.6 mV, $n = 8$, $P = 0.002$, Fig. 3A-B). To measure possible alterations of the input resistance (R_{in}) in response to SST application, we injected negative currents from 0 pA to -100 pA with 20 pA steps for a duration of 600 ms before and during the application of SST when the maximal effect was reached. We fit the current-voltage relationship with a linear function for each cell to obtain R_{in} , which equals the slope of the linear fitting (Fig. 3C-E). Application of SST significantly reduced R_{in} (Control: 91.7 ± 10.4 M Ω , SST: 81.5 ± 12 M Ω , $n = 5$, $P = 0.007$, Fig. 3F left), indicating that SST increased the membrane conductance.

In response to the negative current injections, the voltage responses of the BCs showed an initial peak hyperpolarization and then returned to a steady state (Fig. 3C-D). This is a typical “sag” response indicating the expression of the hyperpolarization-activated channels (Ih channels) in the BCs. As will be shown below, our results demonstrate that SST-

mediated hyperpolarization is mediated by decreasing the activity of AC and intracellular cAMP level. Because I_h channels are facilitated by intracellular cAMP, one possibility is that SST hyperpolarizes subicular neurons by reducing the production of intracellular cAMP to down-regulate I_h channels. If this is the case, application of SST should reduce the sag response as measured by subtracting the peak from the steady state of the hyperpolarization elicited by -100 pA current injection (Fig. 3C-D). However, application of SST failed to significantly alter the sag response (Control: 1.63 ± 0.35 mV, SST: 1.48 ± 0.31 mV, $n=5$, $P=0.41$, Fig. 3F right), suggesting that SST has no significant effects on I_h channels.

SST-induced hyperpolarization is mediated primarily by opening K^+ channels but SST may hyperpolarize some cells by inhibiting cation channels

We next determined the ionic mechanisms whereby SST hyperpolarizes subicular BCs. Because Ca^{2+} is involved in modulating numerous ion channels and SST has been shown to inhibit Ca^{2+} channels (Boehm and Betz, 1997; Farrell et al., 2014), we tested whether extracellular Ca^{2+} is required for SST-induced hyperpolarization by substituting extracellular Ca^{2+} with the same concentration of Mg^{2+} and adding EGTA (0.1 mM) to chelate any potential trace Ca^{2+} . In this situation, bath application of SST still induced a comparable outward HC (40.4 ± 7.9 pA, $n=6$, $P=0.004$ vs. baseline, $P=0.32$ vs. control, Fig. 4A and 4N) suggesting that extracellular Ca^{2+} is not required for SST-induced hyperpolarization. We further determined whether intracellular Ca^{2+} is required for SST-induced hyperpolarization by including BAPTA (10 mM) in the recording pipettes. After the formation of whole-cell recording configuration, we waited for at least 15 min to allow for the diffusion of BAPTA into the cells. Under these circumstances, application of SST induced a statistically indistinguishable outward HC (38.7 ± 7.0 pA, $n=7$, $P=0.001$ vs. baseline, $P=0.39$ vs. control, Fig. 4B and 4N). These results demonstrate that SST-induced hyperpolarization is Ca^{2+} -independent.

We then measured the reversal potential of the currents generated by SST to further determine the involved ionic mechanisms. The extracellular solution contained TTX (1 μ M) and 0 Ca^{2+} to void the contribution of voltage-gated Na^+ and Ca^{2+} channels and the above K^+ -gluconate-containing intracellular solution was used. We used a ramp protocol (from -120 mV to -40 mV) to measure the I-V curve before and during the application of SST. We recorded the I-V curves from 13 cells. Of the 13 cells, 8 cells displayed a linear I-V curve for the SST-generated currents with a reversal potential of -88.7 ± 2.8 mV ($n=8$, Fig. 4C-D), close to the calculated K^+ reversal potential (-89.6 mV), suggesting that SST hyperpolarizes most of the cells by opening K^+ channels. For 4 cells, the I-V curve before and during the application of SST did not intersect between -120 mV and -40 mV (Fig. 4E) and the SST-elicited currents were persistently outward in this voltage range (Fig. 4F), suggesting that SST also hyperpolarizes some cells by inhibiting cation channels. For one cell, the SST-induced currents showed a linear I-V curve but reversed at ~ -60 mV, which was between the K^+ reversal potential and the reversal potential of cations, suggesting that both mechanisms contribute to SST-elicited hyperpolarization in this cell.

If inhibition of cation channels is a mechanism underlying SST-elicited hyperpolarization, the cation channels should open in RMPs. We tested this hypothesis by replacing NaCl in

the extracellular solution with the same concentration of NMDG-Cl. In both conditions, extracellular solution contained TTX (0.5 μ M), kynurenic acid (1 mM) and picrotoxin (100 μ M). Substitution of extracellular NaCl with NMDG-Cl induced an outward HC by itself (31.5 ± 12.1 pA, $n = 6$, $P = 0.048$, Fig. 4G). One reasonable explanation for this result is that a Na^+ -permeant cation channel contributes to the maintenance of RMPs in subicular BCs. However, replacing extracellular Na^+ with NMDG did not significantly alter SST-induced outward HCs (33.7 ± 7.3 pA, $n = 7$, $P = 0.8$ vs. the effect of SST in control condition, Fig. 4G), suggesting that SST-elicited hyperpolarization is principally mediated by the activation of K^+ channels, whereas in some cells SST may also inhibit cation channels.

We further probed the roles of the K^+ channels in SST-induced hyperpolarization. Replacing intracellular K^+ -gluconate with the same concentration of Cs^+ -gluconate blocked SST-induced outward HCs (-0.3 ± 1.9 pA, $n = 5$, $P = 0.87$ vs. baseline, Fig. 4H), further confirming the involvement of K^+ channels. Because SST has been shown to augment GIRK channels in cultured locus coeruleus neurons (Inoue et al., 1988) and possibly in the dorsolateral septal nucleus (Twery and Gallagher, 1989) and micromolar concentration of Ba^{2+} (100-300 μ M) has been shown to block GIRK channels by at least 80% while having little effect on other K^+ channels (Carr and Surmeier, 2007; Lacey et al., 1988; Slugg et al., 1999), we examined the effects of 200 μ M Ba^{2+} on SST-induced outward HCs. Application of 200 μ M Ba^{2+} by itself induced an inward HC (-42.0 ± 6.4 pA, $n = 9$, $P < 0.001$, Fig. 4I), suggesting that GIRK channels are involved in the modulation of RMPs in the BCs. In the presence of Ba^{2+} , application of SST induced a significantly smaller outward HC (10.1 ± 3.3 pA, $n = 9$, $P = 0.02$ vs. baseline, $P = 0.003$ vs. control, Fig. 4I and 4N), suggesting that SST hyperpolarizes BCs by opening GIRK channels. Moreover, application of SCH23390 (20 μ M), another GIRK blocker (Kuzhikandathil and Oxford, 2002), induced an inward HC by itself (-18.7 ± 6.1 pA, $n = 14$, $P = 0.009$, Fig. 4J) and application of SST in the presence of SCH23390 elicited a significantly smaller outward HC (10.2 ± 3.1 pA, $n = 14$, $P = 0.005$ vs. baseline, $P < 0.001$ vs. control, Fig. 4J and 4N). Because intracellular application of QX314 has been shown to block GIRK channels (Alreja and Aghajanian, 1994; Andrade, 1991; Andrade, 1993; Lee and Sherman, 2009), we then tested whether intracellular application of QX314 via the recording pipettes influences SST-induced outward currents. Perfusion of QX314 (625 μ M) into the cells via the recording pipettes significantly reduced SST-induced outward HCs (12.1 ± 3.6 pA, $n = 5$, $P = 0.03$ vs. baseline, $P = 0.04$ vs. control, Fig. 4K and 4N), further supporting the involvement of GIRK channels.

Because KCNQ channels are also involved in SST-induced depression of neuronal excitability in the hippocampus (Moore et al., 1988; Qiu et al., 2008; Schweitzer et al., 1998), we also examined the roles of KCNQ channels in SST-mediated hyperpolarization in subicular BCs. Application of the selective KCNQ channel blocker, linopirdine (30 μ M), induced an inward HC by itself (-24.2 ± 5.4 pA, $n = 16$, $P < 0.001$, Fig. 4L). In the presence of linopirdine, application of SST induced a significantly smaller outward HC (14.1 ± 2.3 pA, $n = 16$, $P < 0.001$ vs. baseline, $P = 0.001$ vs. control, Fig. 4L and 4N). This result suggests that the function of KCNQ channels also contributes to SST-induced hyperpolarization.

We further tried to record I_h channels in voltage-clamp mode from the BCs to test the effects of SST on I_h channels. However, I_h channels showed significant rundown which prevented reliable determination of the actions of SST on I_h channels. We therefore included the specific I_h channel blocker, ZD7288 in the recording pipettes and tested its effects on SST-induced increases in HCs. Consistent with previous results, inclusion of ZD7288 (50 μ M) in the recording pipettes blocked I_h currents recorded from the BCs (Fig. 4M, *Upper*), but failed to significantly reduce SST-induced increases in outward HCs (25.7 ± 5.4 pA, $n = 14$, $P < 0.001$ vs. baseline, $P = 0.32$ vs. control, Fig. 4M-N), further confirming that I_h channels are unlikely to be responsible for SST-induced hyperpolarization of BCs.

If SST generates hyperpolarization by activating the GIRK channels, the currents generated by SST should show inward rectification. However, the currents generated by SST did not show inward rectification at least between -120 mV and -40 mV (Fig. 4D). Because intracellular spermine is required for the exhibition of the inward rectification of GIRK channels (Fakler et al., 1995; Ficker et al., 1994), we examined the I-V curve of SST-elicited currents in the intracellular solution containing spermine (100 μ M). In the presence of spermine, SST-elicited currents showed slight inward rectification ($n = 7$, Fig. 4N-P).

SST₂ receptors are involved in SST-elicited hyperpolarization

We next determined the subtype(s) of SST receptors involved in SST-mediated hyperpolarization by recording HCs at -60 mV. There are 5 types of SST receptors (sst₁-sst₅) (Theodoropoulou and Stalla, 2013). We used the available selective agonists for each subtype of the SST receptors. No significant alteration of HCs was observed in response to the application of the selective agonists for sst₁ (CH275, 100 nM, $K_i = 0.3$ nM, -3.6 ± 4.8 pA, $n = 8$, $P = 0.48$, Fig. 5A and 5G) (Liapakis et al., 1996), sst₄ (L-803,087, 100 nM, $K_i = 0.7$ nM, -3.6 ± 5.4 pA, $n = 11$, $P = 0.53$, Fig. 5D and 5G) (Rohrer and Schaeffer, 2000) and sst₅ (BIM 23052, 100 nM, $K_i = 7.3$ nM, 2.2 ± 1.0 pA, $n = 6$, $P = 0.09$, Fig. 5E and 5G) (Patel and Srikant, 1994). We also used a selective sst₂ agonist, (1R,1'S,3'R/1R,1'R,3'S)-L-054,264 (abbreviated as L-054,264, $K_i = 1.6$ nM for sst₂, 1.74 μ M for sst₁, 2.95 μ M for sst₃, 2.0 μ M for sst₄ and 4.47 μ M for sst₅) (Yang et al., 1998). However, application of 100 nM L-054,264 did not induce an outward HC significantly (4.3 ± 1.7 pA, $n = 5$, $P = 0.06$, data not shown). Because an effective concentration of L-054,264 at 1 μ M in slices has been reported (Vuong et al., 2015), we raised the concentration of L-054,264 to this concentration. Application of 1 μ M L-054,264 reliably induced an outward HC (24.1 ± 7.9 pA, $n = 12$, $P = 0.011$, Fig. 5B and 5G), suggesting that activation of sst₂ receptors induces an outward HC. Similarly, application of octreotide (100 nM), an agonist for sst₂ ($K_d = 0.4 \sim 2.1$ nM), sst₃ ($K_d = 4.4 \sim 34.5$ nM) and sst₅ ($K_d = 5.6 \sim 32$ nM) (Hannon et al., 2002), also induced an outward HC (30.1 ± 8.7 pA, $n = 9$, $P = 0.008$, Fig. 5C and 5G). Application of octreotide at this concentration should mainly activate sst₂ receptors (Boehm and Betz, 1997; Poll et al., 2010). We further used the selective sst₂ antagonist to confirm our results. Application the selective sst₂ antagonist, CYN154806 (2 μ M) blocked SST-induced outward HC (6.7 ± 3.3 pA, $n = 11$, $P = 0.07$ vs. baseline, $P < 0.001$ vs. SST alone, Fig. 5F-G). These results together indicate that SST hyperpolarizes BCs in the subiculum via activation of sst₂ receptors.

G proteins and AC are required for SST-elicited hyperpolarization

We next examined the signaling mechanisms whereby activation of SST receptors hyperpolarizes subicular BCs. Because SST receptors are coupled to the inhibitory G proteins ($G\alpha_i$ and $G\alpha_o$) which inhibit AC resulting in a reduction of cAMP generation and an inhibition of PKA (Theodoropoulou and Stalla, 2013), we tested the roles of this pathway in SST-induced hyperpolarization. Inclusion of the selective G-protein inactivator, GDP- β -S (2 mM) in the recording pipettes blocked SST-elicited augmentation of outward HCs (-3.4 ± 2.6 pA, $n = 6$, $P = 0.25$, Fig. 6A and 6D), demonstrating that G-proteins are required for SST-mediated hyperpolarization.

We then tested the requirement of AC. Slices were pretreated with the selective AC inhibitor, MDL12330A (30 μ M), for 30 min. Application of SST to the pretreated slices did not elicit a significant outward HC (3.2 ± 3.0 pA, $n = 13$, $P = 0.31$ vs. baseline, Fig. 6B and 6D). Likewise, pretreatment of slices with another selective AC inhibitor, SQ22536 (500 μ M), for 30 min significantly reduced SST-evoked outward HCs (9.5 ± 3.1 pA, $n = 8$, $P = 0.02$ vs. baseline, $P = 0.004$ vs. control, Fig. 6C and 6D). These data together demonstrate that AC is required for SST-mediated increases in outward HCs.

If SST hyperpolarizes subicular neurons via G_i -protein-mediated inhibition of AC and decreased level of intracellular cAMP, elevation of intracellular cAMP concentration endogenously should depolarize subicular neurons. Consistent with this notion, bath application of forskolin (25 μ M), an AC activator, induced an inward HC (-32.2 ± 4.2 pA, $n = 6$, $P < 0.001$ vs. baseline, Fig. 6E and 6H) in the BCs of the subiculum. Similarly, bath application of IBMX (250 μ M), an inhibitor of phosphodiesterase which degrades intracellular cAMP, induced an inward HC (-35.2 ± 3.4 pA, $n = 11$, $P < 0.001$, Fig. 6F and 6H). These results together demonstrate that intracellular cAMP exerts a tonic control over RMPs in the subiculum.

Our result that SST acts by inhibiting AC to lower intracellular cAMP level suggests that a certain level of AC activity and cAMP concentration is essential to witness the effect of SST. We reasoned that prior increases in the basal level of cAMP should set a higher baseline and enhance the effect of SST. We therefore used IBMX as an example to test this idea. When the effect of IBMX on the inward HCs reached the maximal level, we applied SST in the continuous presence of IBMX. Under these circumstances, application of SST induced a significantly larger outward HC (49.6 ± 5.8 pA, $n = 10$, $P < 0.001$ vs. baseline, $p = 0.011$ vs. control, Fig. 6G and 6H). This result further supports the hypothesis that SST hyperpolarizes subicular neurons by lowering intracellular cAMP level.

PKA and PKC are not required for SST-mediated hyperpolarization

We further tested the roles of PKA by using a variety of PKA inhibitors. We included the specific PKA inhibitor, Rp-cAMPS (200 μ M), in the recording pipettes and waited for >20 min after formation of the whole-cell configuration to allow the drug to diffuse into the recorded cells. In the presence of Rp-cAMPS, application of SST still induced a comparable outward HC (25.6 ± 7.3 pA, $n = 6$, $P = 0.017$ vs. baseline, $P = 0.48$ vs. control, Fig. 7A and 7G). Application of PKI (5-24) (1 μ M), another selective PKA inhibitor, in the same fashion,

failed to block SST-elicited outward HCs (26.1 ± 5.7 pA, $n = 7$, $P = 0.004$ vs. baseline, $P = 0.48$ vs. control, Fig. 7B and 7G). Furthermore, pretreatment of slices with H89 (10 μ M), another selective PKA inhibitor, for >2h and continuous intracellular presence of H89 via the recording pipettes failed to block SST-induced outward HCs (37.4 ± 9.4 pA, $n = 7$, $P = 0.007$ vs. baseline, $P = 0.49$ vs. control, Fig. 7C and 7G). Likewise, application of KT5720 (2 μ M) in the same fashion as H89 did not significantly alter SST-induced outward HCs (26.8 ± 5.5 pA, $n = 7$, $P = 0.003$ vs. baseline, $P = 0.54$ vs. control, Fig. 7D and 7G). These results together demonstrate that PKA is unnecessary for SST-induced outward currents.

Because SST receptors also signal via PKC (Cervia et al., 2008; Pittaluga et al., 2000; Pittaluga et al., 2005), we tested whether PKC is involved in SST-elicited hyperpolarization. Slices were pretreated with GF 109203X (2 μ M), a broad-spectrum PKC inhibitor, and the same concentration of GF 109203X was included in the recording pipettes. In the presence of GF 109203X, application of SST still induced a comparable outward HC (24.5 ± 5.9 pA, $n = 9$, $P = 0.003$ vs. baseline, $P = 0.32$ vs. control, Fig. 7E and 7G), suggesting that PKC is unnecessary for SST-mediated hyperpolarization.

Epac2 is partially involved in SST-elicited hyperpolarization

Increasing evidence suggests that intracellular cAMP functions by interacting with Epac (Gloerich and Bos, 2010) which includes Epac1 and Epac2. Epac1 is expressed in most non-neural tissues and is far less abundant in the brain, whereas Epac2 is highly enriched in the brain, especially in the cortex and hippocampus (Kawasaki et al., 1998). We therefore tested the roles of Epac2 by using a selective Epac2 inhibitor, HJC0350 (Chen et al., 2013). In the presence of HJC0350 (100 μ M), SST-elicited an outward HC that was significantly reduced (7.9 ± 4.0 pA, $n = 13$, $P = 0.08$ vs. baseline, $P < 0.001$ vs. control, Fig. 7F and 7G), demonstrating that Epac2 is partially involved in SST-mediated hyperpolarization.

SST decreases AP firing frequency of the BCs

We next examined the effects of SST-mediated hyperpolarization on AP firing. Because subicular neurons do not exhibit spontaneous AP firing, we injected a positive current to evoke a basal AP firing at a frequency of 2-7 Hz. Under these circumstances, bath application of SST (1 μ M) for 5 min inhibited AP firing frequency to $6 \pm 4\%$ of control (Control: 3.88 ± 0.55 Hz, SST: 0.25 ± 0.19 Hz, $n = 8$, $P < 0.001$, Fig. 8A-B). The inhibitory effect of SST was partially reversed after wash for 10 min (1.73 ± 0.69 Hz, $42 \pm 13\%$ of control, $n = 8$, Fig. 8A-B).

SST does not modulate synaptic transmission but depresses LTP

We further examined the potential effects of SST on synaptic transmission and LTP. Application of SST for 5 min did not significantly affect AMPA EPSCs recorded at the CA1-BC synapses ($99 \pm 7\%$ of control, $n = 9$, $P = 0.88$, Fig. 8C) but significantly ($P = 0.017$, two-way ANOVA) reduced LTP ($126 \pm 5\%$ of control, $n = 10$, $P < 0.001$ vs. baseline, Fig. 8D-F) compared with the slices treated with saline ($161 \pm 8\%$ of control, $n = 10$, $P < 0.001$ vs. baseline, Fig. 8E-F), demonstrating that SST exerts an inhibitory effect on LTP at the CA1-BC synapses. Because SST hyperpolarizes postsynaptic BCs, we then tested whether SST-induced hyperpolarization is responsible for its inhibitory action on LTP. We

recorded stable AMPA EPSCs for 5 min and then applied SST for 5 min in current clamp because SST-induced maximal hyperpolarization was usually observed within 5 min after the beginning of SST application. We injected a positive current to bring the membrane potential back to the initial level prior to SST application and then applied HFS in current clamp. Under these circumstances, SST-induced depression of LTP was annulled ($171 \pm 11\%$ of control, $n = 8$, $P < 0.001$ vs. baseline, $P = 0.5$ vs. slices treated with saline, Fig. 8F). These results together demonstrate that SST-elicited hyperpolarization of BCs is responsible for its inhibitory effect on LTP at the CA1-BC synapses.

Discussion

Whereas SST has been demonstrated to hyperpolarize both BCs and RCs (Greene and Mason, 1996a; Greene and Mason, 1996b) and reduce both GABA_A receptor-mediated fast IPSPs and GABA_B receptor-induced slow IPSPs (Greene and Mason, 1996b) in the subiculum, the underlying cellular and molecular mechanisms of SST have not been determined. In the present study, we focus on elucidating the mechanisms whereby SST decreases the excitability of the BCs because this is the predominant type of neurons in the subiculum. As schematically illustrated in Fig. 9, our results demonstrate that activation of sst₂ receptors hyperpolarizes subicular BCs via opening K⁺ channels, including GIRK and KCNQ channels, and may also inhibit cation channels in some cells. SST-elicited hyperpolarization requires the function of G proteins and is mediated by inhibiting AC and lowering intracellular cAMP concentration. PKA and PKC are not involved in SST-mediated depression of neuronal excitability in the subiculum, whereas Epac2 is partially required. SST depresses AP firing frequency and inhibits LTP via hyperpolarizing postsynaptic BCs at the CA1-BC synapses, suggesting that SST modulates subicular network function.

Our results demonstrate that SST hyperpolarizes subicular neurons via activation of K⁺ channels. However, the type of the involved K⁺ channels has not completely been identified. There are two candidates for the involved K⁺ channels. The first one is GIRK channels because application of low concentration of Ba²⁺ (200 μ M) or SCH23390, another GIRK blocker (Kuzhikandathil and Oxford, 2002), significantly reduced SST-mediated outward HCs. However, the I-V curve of SST-induced net currents did not show inward rectification at least between -120 mV and -40 mV. Because spermine or spermidine is required to maintain the inwardly rectifying character of GIRK channels (Ficker et al., 1994), we also recorded the I-V curves in the intracellular solution containing spermine (100 μ M). Under these circumstances, the I-V curve of the SST-induced currents showed slight inward rectification. One explanation for the discrepancy of electrophysiological and pharmacological properties of the SST-activated K⁺ channels is that SST-activated GIRK channels belong to the subtypes of weak inward rectification (Bredt et al., 1995; Doupnik et al., 1995). The second candidate for the K⁺ channels activated by SST is KCNQ channels because application of the selective KCNQ channel blocker, linopirdine, significantly decreased SST-elicited outward HCs. Given the fact that the activation voltage threshold of KCNQ is around or positive to the RMPs of the neurons (Halliwell and Adams, 1982), the contribution of KCNQ channels to the RMPs is limited. Consistent with this idea, our results demonstrate that KCNQ channels are only partially responsible for SST-mediated outward HCs at -60 mV which is close to the RMP of these neurons. However, KCNQ channels

would be expected to contribute more to the inhibitory effects of SST during the phase of action potential firing, as the neurons are depolarized to the activation range of these channels.

Whereas our results demonstrate that SST hyperpolarizes subicular BCs primarily by activating K^+ channels, an inhibition of a cation conductance is also implicated in SST-elicited inhibition of subicular neuronal excitability because in some cells SST-elicited net currents did not reverse in the voltages ranging from -120 mV to -40 mV. However, several lines of evidence do not support a role of cation channels in SST-mediated hyperpolarization. First, application of SST failed to evoke significant increases in HCs when intracellular K^+ was replaced with Cs^+ . Second, substitution of extracellular NaCl with the same concentration of NMDG-Cl did not significantly alter SST-elicited enhancement of HCs. Third, if cation channels are involved, I_h channels are an excellent candidate because I_h channels are up-regulated by intracellular cAMP and our results demonstrate that SST-mediated hyperpolarization of BCs is due to a reduction of intracellular cAMP concentration. However, SST did not significantly change the sag response recorded in current clamp and inclusion of the specific I_h channel blocker, ZD7288, in the recording pipettes failed to significantly reduce SST-induced increases in HCs. For those cells exhibiting a persistent outward current from -120 mV to -40 mV in response to SST, the voltage-current relationship of the net currents evoked by SST does not resemble that of the I_h channels (Fig. 4F). We therefore conclude that I_h channels are not involved in SST-induced hyperpolarization in the subicular BCs. Whereas the cation channels involved in SST-induced hyperpolarization need further identification, consistent with our results, application of opioid peptide met-enkephalin suppresses a resting Na^+ -permeant inward current and activates an outward K^+ current in locus coeruleus neurons (Alreja and Aghajanian, 1993).

Whereas SST activates 5 subtypes of SST receptors, our results demonstrate that activation of sst_2 is required for SST-elicited hyperpolarization based on the experiments with the selective agonists and antagonist. Whereas G protein-coupled receptors can exert functions via G protein-independent mechanisms, our results demonstrate that G proteins are required for sst_2 -mediated outward HCs because intracellular application of the selective G protein inactivator, GDP- β -S, completely blocked SST-induced hyperpolarization. Because one of the major signals coupled to sst_2 is AC, we tested the roles of AC in SST-mediated outward HCs. Our results demonstrate that AC is necessary for the effect of SST. The requirement of AC in SST-induced outward HCs suggests that the RMPs of the BCs in the subiculum are tonically controlled by the intracellular cAMP level. This notion is further confirmed by the results that application of the reagents to endogenously elevate intracellular cAMP level including forskolin and IBMX induced an inward HC. There are at least three possibilities for intracellular cAMP to alter RMPs. First, cAMP activates PKA resulting in phosphorylation of ion channels. However, application of the selective PKA inhibitors including Rp-cAMPS, H89, PKI(5-24) and KT5720 failed to change SST-elicited outward HCs, demonstrating that PKA is unnecessary for SST-elicited hyperpolarization. Second, recent studies reveal that Epac is another target for intracellular cAMP (Gloerich and Bos, 2010) and Epac2 is highly enriched in the brain (Kawasaki et al., 1998). We therefore tested the roles of Epac2 and found that application of a selective Epac2 inhibitor, partially blocked

SST-mediated outward HCs, suggesting that Epac2 is at least partially responsible for SST-mediated hyperpolarization. Consistent with our results, activation of SST receptors inhibits Epac (Jacobs et al., 2010) and Epac inhibits GIRK channels in medial prefrontal cortex pyramidal neurons (Witkowski et al., 2012). Third, cAMP by itself can interact with ion channels to alter RMPs. Because application of the Epac2 inhibitor only partially blocked SST-elicited outward HC, the possibility that cAMP directly interacts with ion channels to mediate SST-elicited hyperpolarization cannot be excluded.

In the brain, SST is synthesized in and released by GABAergic neurons. The phasic release of SST by local interneurons likely modulates the functions of neural network activities. SST has been reported to enhance the late phase of LTP in apical and basal dendrites in CA1 region (Fan and Fu, 2014) but depresses LTP in the dentate gyrus possibly by inhibiting N-type Ca^{2+} channels (Baratta et al., 2002). Our results demonstrate that SST-elicited hyperpolarization underlies its inhibitory effect on LTP at the CA1-BC synapses in the subiculum. Because LTP is generally considered as a cellular model for learning and memory and the subiculum also participates in the formation of a cognitive map encoding spatial, contextual, and emotional information which is then transmitted throughout the brain (O'Mara et al., 2001), it is reasonable to speculate that SST-elicited hyperpolarization and depression of LTP in the subiculum contribute to its effects on learning and memory. Consistent with our conjecture, SST inhibits learning and memory (Borbely et al., 2013) and knocking out *sst2* enhances spatial learning ability in mice (Dutar et al., 2002). Furthermore, the subiculum is an important structure in the limbic system which controls emotional responses. Given that anxiety is related to an elevation of glutamatergic functions (Bergink et al., 2004) and because the subicular BCs are glutamatergic, SST-mediated hyperpolarization of subicular neurons likely depresses the glutamatergic functions in the neural network activity of the limbic system, resulting in anxiolytic actions. In line with our anticipation, activation of somatostatinergic system generates anxiolytic-like actions (Engin et al., 2008; Engin and Treit, 2009; Yeung et al., 2011; Yeung and Treit, 2012). It appears that *sst2* receptors are responsible for SST-mediated anxiolytic effects as demonstrated by pharmacological experiments (Engin and Treit, 2009; Yeung and Treit, 2012) and *sst2* knockout mice (Viollet et al., 2000). Moreover, the limbic system is intimately associated with epilepsy and there is compelling evidence demonstrating that SST exerts robust antiepileptic actions although previous efforts were focused on the hippocampus (Tallent and Qiu, 2008; Tallent and Siggins, 1999). Because the subiculum is an indispensable structure for the completion of hippocampal functions, SST-elicited depression of the excitability of subicular neurons likely contributes to its antiepileptic actions. Collectively, our results provide a cellular and molecular mechanism at least partially to explain the physiological functions and pathological roles of somatostatinergic system in the brain.

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Fig. 1.

Photo taken under a microscope from a horizontal slice to show the location of subiculum (Sub) encircled by the dashed line. Recordings were conducted from the pyramidal neurons in the middle 1/3 of the subiculum. Stimulation electrode was placed in the alveus of the border of CA1 and subiculum. DG: dentate gyrus

Fig. 2.

Bath application of SST induces an outward HC in both BCs and RCs. **A**, SST induced an outward HC in a BC. *Upper*: Voltage response elicited by injection of ± 100 pA in a BC. Inset shows the enlarged bursting of APs. *Lower*: Outward HC evoked by SST from the same BC. **B**, SST induced an outward HC in a RC. *Upper*: Voltage response elicited by injection of ± 100 pA in a RC. Inset shows the enlarged single AP. *Lower*: Outward HC evoked by SST from the same RC. **C**, Net HCs elicited by SST from BCs ($n=58$) and RCs ($n=11$). Open symbols represent values from individual cells and the solid symbols are their averages. **D**, Concentration-response curve. Numbers in the parentheses are the numbers of BCs recorded for each concentration.

Fig. 3.

SST hyperpolarizes subicular neurons and decreases input resistance (R_{in}). **A**, Application of SST induced hyperpolarization of a subicular neuron. **B**, Summary graph for SST-induced hyperpolarization ($n=8$). **C**, Voltage responses evoked by injection of negative currents from -100 pA to 0 pA at an interval of 20 pA in control condition. Arrows indicate the measurement of peak hyperpolarization and steady state, respectively. Sag response is defined as the subtraction of the peak from the steady state. **D**, Voltage responses evoked by injection of negative currents from -100 pA to 0 pA at an interval of 20 pA from the same cell in the presence of SST. **E**, Current-voltage relationship averaged from 5 cells. R_{in} was obtained by linear fitting of the current-voltage relationship. **F**, Summary graph for R_{in} (*left*) and sag (*right*) before and during the application of SST ($n=5$).

Fig. 4. Multiple ionic mechanisms underlying SST-mediated hyperpolarization of BCs in subiculum. **A**, Deprivation of extracellular Ca^{2+} did not block SST-induced outward HCs. **B**, Inclusion of BAPTA (10 mM) in the recording pipettes failed to block SST-elicited outward HCs. **C**, I-V curves recorded before and during the application of SST averaged from 8 cells. **D**, I-V curve of the net current by subtracting the traces recorded in control condition (I_{Control}) from those recorded in the presence of SST (I_{SST}). **E**, I-V curves recorded before and during the application of SST averaged from 4 cells. **F**, I-V curve of the net current by subtracting the traces recorded in control condition (I_{Control}) from those recorded in the presence of SST (I_{SST}). **G**, Replacing extracellular NaCl with NMDG-Cl in the continuous presence of TTX elicited an outward HC and subsequent application of SST still evoked an outward HC. **H**, Replacement of intracellular K^{+} -gluconate with the same concentration Cs^{+} -gluconate blocked SST-evoked augmentation of outward HCs. **I**, Inclusion of 200 μM Ba^{2+} in the extracellular solution induced an inward HC by itself and reduced SST-elicited outward HCs. **J**, Inclusion of SCH23390 (20 μM) in the extracellular solution induced an

inward HC by itself and reduced SST-evoked outward HCs. **K**, Inclusion of QX314 (625 μM) in the recording pipettes reduced SST-elicited outward HCs. **L**, Inclusion of linopirdine (30 μM) in the extracellular solution evoked an inward HC and attenuated SST-mediated outward HCs. **M**, Inclusion of ZD7288 (50 μM) in the recording pipettes blocked I_h currents (*upper*), but did not block SST-induced outward HC (*Lower*). The I_h currents were evoked by hyperpolarization from -50 mV to -120 mV for 1 s from a cell recorded with normal intracellular solution (*left*) and a cell with the intracellular solution containing ZD7288 (50 μM , *right*). ZD7288-mediated blocking of I_h currents was so fast that very little I_h currents left just after the formation of whole-cell configuration preventing showing current traces from the same cells before and after blockade of I_h by ZD7288. **N**, Summary graph. Empty symbols represent the net currents from individual cells and filled symbols are their averages. **O**, I-V curves recorded before and during the application of SST averaged from 7 cells in the intracellular solution containing spermine (100 μM). **P**, I-V curve of the net current by subtracting the traces recorded in control condition (I_{Control}) from those recorded in the presence of SST (I_{SST}) in the intracellular solution containing spermine (100 μM).

Fig. 5.

SST hyperpolarizes subicular BCs via activation of sst_2 receptors. **A**, Bath application of the selective sst_1 agonist (CH 275, 100 nM) did not change HCs. **B**, Application of the selective sst_2 agonist (L-054,264, 1 μ M) induced an outward HC. **C**, Application of another sst_2 agonist (octreotide, 100 nM) induced an outward HC. **D**, Application of the selective sst_4 agonist (L-803,087, 100 nM) did not elicit an outward HC. **E**, Application of the selective sst_5 agonist (BIM 23052, 100 nM) failed to induce an outward HC. **F**, Bath application of the selective sst_2 antagonist (CYN154806, 2 μ M) blocked SST-mediated outward HCs. **G**, Summary graph. * $p < 0.05$, ** $P < 0.01$ vs. baseline.

Fig. 6.

G proteins and AC are necessary for SST-mediated outward HCs. **A**, Application of GDP-β-S (2 mM) blocked SST-mediated outward HCs. **B**, Pretreatment of slices with MDL 12330A (30 μM) decreased SST-mediated outward HCs. **C**, Pretreatment of slices with SQ 22536 (500 μM) decreased SST-elicited outward HCs. **D**, Summary graph. * $p < 0.05$ vs. baseline. **E**, Bath application of forskolin (25 μM) induced an inward HC in a subicular BC. **F**, Bath application of IBMX (250 μM) induced an inward HC in a subicular BC. **G**, Bath application of IBMX induced an inward current by itself and application of SST elicited a larger outward HC in the continuous presence of IBMX. **H**, Summary graph.

Fig. 7.

PKA and PKC are not required for SST-elicited outward HCs, but Epac2 is partially involved. **A**, Application of Rp-cAMPS (200 μ M) via the recording pipettes did not block SST-mediated outward HCs. **B**, Inclusion of PKI(5-24) (2 μ M) in the recording pipettes failed to alter SST-induced outward HCs. **C**, Pretreatment of slices with and inclusion of H89 (10 μ M) in the recording pipettes did not block SST-evoked outward HCs. **D**, Pretreatment of slices with and inclusion of KT 5720 (5 μ M) in the recording pipettes did not block SST-elicited outward HCs. **E**, Pretreatment of slices with and inclusion of GF109203X (2 μ M) in the recording pipettes failed to block SST-elicited outward HCs. **F**, Pretreatment of slices with and inclusion of HJC0350 (100 μ M) in the recording pipettes reduced SST-induced outward HCs. **G**, Summary graph.

Fig. 8.

SST depresses AP firing frequency and LTP. **A**, APs recorded from a BC prior to, during and after the application of SST. **B**, Averaged time course of SST-mediated depression of AP firing frequency (n = 8). **C**, Averaged time course of AMPA EPSC amplitudes in response to bath application of SST (n = 9). Insets show the average AMPA EPSCs at the time points indicated by the arrows. **D**, Time course of LTP at a CA1-BC synapse elicited by a HFS protocol applied after perfusion of SST for 5 min. Inset shows the AMPA EPSCs recorded at the time points indicated in the figure. **E**, Time course of LTP at a CA1-BC synapse elicited by the same HFS protocol applied after perfusion of saline (0.9% NaCl) for 5 min. Inset shows the AMPA EPSCs recorded at the time points indicated in the figure. **F**, Averaged LTPs in response to bath application of saline, SST and SST plus positive current injection during the application of HFS protocol.

Fig. 9. Schematic diagram of proposed sst₂ signaling pathway involved in SST-induced hyperpolarization of subicular BCs. SST activates sst₂ receptors resulting in activation of the inhibitory G-proteins (Gα_i). Activation of Gα_i inhibits the activity of adenylyl cyclase (AC) leading to a reduction of intracellular cAMP level from ATP and a decrease of the function of Epac2. The downregulation of cAMP/Epac2 activity results in activation of K⁺ channels including GIRK and KCNQ channels and/or tonic inhibition of a Na⁺-permeable cation channel to hyperpolarize subicular neurons.